

Research Article

Effects of Seasons on Prevalence of Bacterial Isolates in The Borehole Waters in Onitsha, Nigeria

¹Chikodili G. Anaukwu, ²Chisom Peace Okonkwo, ³Chibuikwe G. Obi, ⁴Chukwuemeka Marcellinus Ezeuko

^{1,2,3,4}Department of Applied Microbiology and Brewing, Nnamdi Azikiwe University, Awka, Nigeria

Abstract:

In Onitsha, borehole water serves as the primary source of drinking water for most residents. However, many boreholes are located close to open solid waste dumpsites, raising concerns about bacterial contamination, particularly during seasonal rainfall which can exacerbate the risk increasing the risk. This study examined the effects of seasons on prevalence of bacteria in borehole waters in Onitsha. Water samples were collected aseptically after flushing taps for 2 minutes. Isolation and identification of bacterial organisms were carried out using nutrient agar and selective media including MacConkey, EMB, TCBS, SSA, and Cetrimide. Isolates were identified based on colonial and biochemical characteristics. A total of 126 isolates were recovered and identified as *Escherichia coli*, *Vibrio cholerae*, *Vibrio parahaemolyticus*, *Pseudomonas*, *Salmonella*, and *Shigella* species. Out of the total isolates, 55.6% were recorded in the rainy season, while 44.4% were recovered in dry season. *E. coli* occurred highest in both rainy and dry season at 27.14% and 25% respectively, whereas other isolates except *Shigella* spp, were more prevalent during the rainy season. These findings indicate that borehole water in Onitsha is bacteriologically contaminated and unsafe for direct consumption without treatment.

Keywords borehole water, seasonal variation, Onitsha, bacteria.

Introduction

Access to safe drinking water remains a major challenge in many Nigerian urban centers, where municipal supplies are inadequate or intermittent (WHO & UNICEF, 2021). Consequently, borehole water has become a critical source of potable water in cities such as Onitsha, Nigeria, where residents rely heavily on privately constructed groundwater sources to meet daily domestic needs. However, the close proximity of many boreholes to open solid waste dumpsites poses a significant risk of bacterial contamination, as leachates from decomposing waste can percolate into groundwater and introduce pathogenic organisms (Ubechu et al., 2021). Seasonal variations further exacerbate this risk in tropical regions such as Southeastern Nigeria, which experience distinct wet and dry seasons (Agbasi et al., 2023). During the rainy season (typically April–October), intense precipitation increases surface runoff from dumpsites, transporting fecal matter, organic waste, and bacteria into shallow aquifers through seepage from gutters, soakaways, and poorly managed drainage systems. This often leads to elevated bacterial loads, as rainwater facilitates contaminant transport (Agbasi et al., 2023; Ebong et al., 2018). In contrast, the dry season (November–March) may concentrate pollutants due to reduced dilution and lower groundwater recharge, although infiltration from surface sources is generally reduced (Yan et al., 2025). Similar observations from urban areas such as Port Harcourt have shown higher coliform counts in borehole water during rainy periods, reflecting increased microbial transport associated with rainfall and highlighting the need for bacterial biodiversity assessments (Kumpel et al., 2017). Similar patterns have been observed in other parts of Southeastern Nigeria, where poorly managed dumpsites and inadequate sanitation infrastructure facilitate the transport of fecal indicators and opportunistic pathogens into groundwater, particularly during the wet season (Kumpel et al., 2017). Despite the widespread dependence on boreholes in Onitsha, limited attention has been paid to the composition of bacterial communities in these water sources, particularly those located near open dumpsites, and even less is known about how seasonal variations influence the diversity and abundance of recovered bacteria.

Common bacterial contaminants reported in polluted Nigerian groundwater include enteric organisms such as *Escherichia coli*, *Salmonella* spp., *Shigella* spp., *Vibrio* spp., and *Pseudomonas aeruginosa*, many of which serve as indicators of fecal pollution or direct agents of waterborne disease (Odonkor & Ampofo, 2013; Ebong et al., 2018). The presence of these genera signals significant public health risks, ranging from acute diarrheal illnesses to severe infections among vulnerable populations, particularly children and immunocompromised individuals (WHO, 2017). Understanding not only their occurrence but also their seasonal dynamics is therefore critical for assessing exposure risks and guiding effective intervention strategies.

This study therefore aimed to characterize the bacterial diversity of borehole water collected near open dumpsites in Onitsha metropolis. Specifically, it sought to isolate and identify bacterial species during both dry and rainy seasons, determine their relative prevalence, and evaluate whether seasonal variations influence bacterial load and community composition. By documenting these

patterns, the study provides practical evidence to guide community-level water safety measures and support improved regulation of borehole siting and waste management in rapidly growing urban areas.

Materials and Methods

Study Area

This study was conducted in Onitsha metropolis, Anambra State, Southeastern Nigeria, located at latitude 6°10'N and longitude 6°47'E. Onitsha lies along the western bank of the River Niger and is divided into two local government areas: Onitsha North and Onitsha South. The city has a tropical climate with a distinct rainy season (April–October) characterized by heavy precipitation and a dry season (November–March) marked by harmattan dust and low rainfall. Topographically, the area features low-lying plains and gentle slopes that drain toward the Niger River, facilitating surface runoff during rains. The population consists predominantly of traders, civil servants, and artisans, with high residential density, numerous markets, motor parks, schools, petrol stations, and other commercial activities that generate large volumes of municipal solid waste. Open dumpsites are common along streets and near residential zones, and many privately owned boreholes often accessible to the public are sited within 50 meters of these dumps, increasing the potential for leachate infiltration into shallow groundwater.

Sample Collection

A total of 40 borehole water samples were collected from 20 different boreholes (10 in Onitsha North and 10 in Onitsha South) located less than 50 m from open solid waste dumpsites. Samples were taken during both the rainy and dry seasons to allow direct seasonal comparison. At each site, the borehole tap was first turned on and allowed to run freely for 2 minutes to flush out stagnant water and obtain a representative sample from the aquifer. Water was then collected aseptically into sterile 500 mL screw-capped plastic bottles, immediately labeled with the date, site code (prefix “ON” for Onitsha North and “OS” for Onitsha South), and season, and transported on ice to the Microbiology Laboratory, Nnamdi Azikiwe University, Awka, for bacteriological analysis within 24 hours.

Enumeration and Isolation of Bacterial Organisms

Heterotrophic bacterial organisms were isolated using the pour-plate technique. One milliliter of the borehole water sample (or appropriate ten-fold serial dilution, typically 10^{-2} and 10^{-3}) was inoculated into sterile nutrient agar plates and incubated at 30°C for 24 hours. Distinct colonies that developed were counted using a colony counter and results expressed as the mean of triplicate plates in Colony-forming units (CFU/mL). The colonies were sub-cultured on fresh nutrient agar plate, MacConkey agar plate, Eosin methylene blue (EMB) agar plate, thiosulphate citrate bile salt (TCBS) agar plate, salmonella-shigella agar (SSA) plate, reinforced differential clostridial (RDC) agar plate and cetrimide agar plates by streaking method to obtain pure bacterial culture and distinguish enteric isolates. The isolates were stored on nutrient agar slants at 4°C for further characterization.

Characterization and Identification of Isolates

Purified isolates were identified based on colonial morphology, Gram staining, and a battery of biochemical tests following standard protocols (Cheesbrough, 2010). Gram staining involved heat-fixed smears stained with crystal violet, Lugol’s iodine, acetone-alcohol decolorization, and safranin counterstain, examined under oil immersion ($\times 100$).

Biochemical tests included: catalase (effervescence with 3% H_2O_2), oxidase (color change on tetramethyl-p-phenylenediamine-soaked filter paper), indole (red ring with Kovac’s reagent in tryptophan broth), methyl red (red color in peptone water after methyl red addition), Voges-Proskauer (red color with naphthol and KOH in glucose broth), citrate utilization (blue color on Simmons citrate agar), urease (pink color in urea broth), hydrogen sulfide production (black precipitate in triple sugar iron agar), motility (diffuse growth in stabbed nutrient agar), and sugar fermentation (acid/gas production in peptone water with glucose, maltose, sucrose, lactose, galactose, fructose, mannitol, and dextrose, using Durham tubes).

Identification to genus level was achieved by comparing results with standard reference charts and keys.

Data Analysis

All tests were performed in triplicate. Prevalence of each genus was expressed as a percentage of total isolates recovered per season.

Results and Discussion

A total of 126 bacterial isolates were recovered from the 40 borehole water samples collected in Onitsha during the rainy and dry seasons. The isolated organisms exhibited consistent Gram-negative rod morphology and varied in biochemical profiles. As presented in Table 1, biochemical tests identified the presence of *Vibrio cholerae*, *Escherichia coli*, *Vibrio parahaemolyticus*, *Pseudomonas aeruginosa*, *Salmonella* spp., and *Shigella* spp., all of which are organisms of significant public health concern (Nkemnyi et al., 2023). The identification of these genera using standard morphological and biochemical tests aligns with established conventional schemes and supports the reliability of the results. Positive catalase reactions across all isolates suggest aerobic or facultatively anaerobic metabolic capacity, which enhances their survival in oxygenated groundwater environments.

Table 1: Biochemical Characteristics of the Bacterial Isolates from the Borehole Water Samples

Isolate	Colony Morphology	Gram Reaction	Catalase	Coagulase	Citrate Utilization	Urease	Indole	Oxidase	Motility	Voges Proskauer	Methyl Red	Sugar Fermentation								Probable Isolates
												Glc	Mal	Lac	Suc	Gal	Frc	Mann	Dex	
1	Yellow colonies on TCBS	-ve comma shape	+	-	+	-	+	+	+	-	-	A G	A G	A	A G	A G	A G	AG	AG	<i>Vibrio cholerae</i>
2	Green metallic sheen on EMB Agar	-ve Rods	+	-	-	-	+	-	+	-	+	A G	A G	A G	A G	A G	A G	AG	AG	<i>Escherichia coli</i>
3	Yellow-green colonies on TCBS	-ve curved Rods	+	-	-	+	+	+	+	-	+	A G	A G	-	-	-	-	AG	AG	<i>Vibrio parahaemolyticus</i>
4	Lemon-Green colonies on CA	-ve Rods	+	-	+	-	-	+	+	-	-	-	-	-	-	-	A G	AG	AG	<i>Pseudomonas aeruginosa</i>
5	Translucent smooth black round colonies on SSA	-ve Rods	+	-	-	-	-	-	+	-	+	A G	A G	-	-	A G	A G	AG	AG	<i>Salmonella</i> spp
6	Round colourless colonies on SSA	-ve Rods	+	-	-	-	+	-	-	-	+	A G	A	-	-	A G	A G	AG	AG	<i>Shigella</i> spp

Key: + = Positive; - = Negative; A+ = Positive with Acid production only; AG = Positive with Acid and Gas production; Lac = Lactose; Frc = Fructose; Mal = Maltose; Suc = Sucrose; Gal = Galactose; Mann = Mannitol; Dex = Dextrose; Glc = Glucose

Figure 1 illustrates the seasonal distribution of bacterial isolates recovered from borehole water samples in Onitsha. Of the total 126 isolates obtained, 70 (55.6%) were recorded during the rainy season, while 56 (44.4%) were recovered during the dry season. This distribution demonstrates a clear seasonal influence on the bacteriological quality of borehole water, with higher bacterial occurrence during the rainy season. The increased proportion of isolates in the rainy season can be attributed to enhanced rainfall-driven contamination processes. During periods of heavy rainfall, surface runoff and leachate from nearby dumpsites, pit latrines, septic tanks, and drainage channels are more likely to infiltrate shallow aquifers (Rahim et al., 2025). In urban areas such as Onitsha, where many boreholes are sited close to open waste dumps and lack adequate sanitary protection, rainfall facilitates the transport of fecal matter and organic debris into groundwater systems. This mechanism explains the higher recovery of bacterial isolates observed in the rainy season.

Similar seasonal trends have been reported in other parts of Nigeria. In Port Harcourt, Ebong et al. (2018) reported significantly

bacterial load in groundwater located near urban waste disposal sites during the wet season, attributing this to enhanced infiltration and surface runoff. Comparable findings were reported in Abakaliki, where peri-urban boreholes exhibited increased bacterial contamination following rainfall due to poor drainage systems and waste seepage (Uhuo et al., 2014). Studies from other parts of southern Nigeria, including Bayelsa and Rivers States, likewise indicate that microbial contamination of borehole water intensifies during the rainy season as a result of leaching from dumpsites and inadequate sanitation infrastructure (Enetimi et al., 2020). Taken together, these observations suggest that the results of the present study reflect a broader pattern associated with rapid urbanization and ineffective waste management.

The relatively small difference between rainy and dry season proportions (Figure 1) also highlights the continuous exposure of residents to bacteriologically unsafe water. While the rainy season poses a higher risk due to intensified contamination pathways, reliance on untreated borehole water during the dry season still carries significant health risks (Kumpel et al., 2017). This finding reveals the importance of year-round water quality monitoring and the need for consistent water treatment practices rather than seasonal interventions alone.

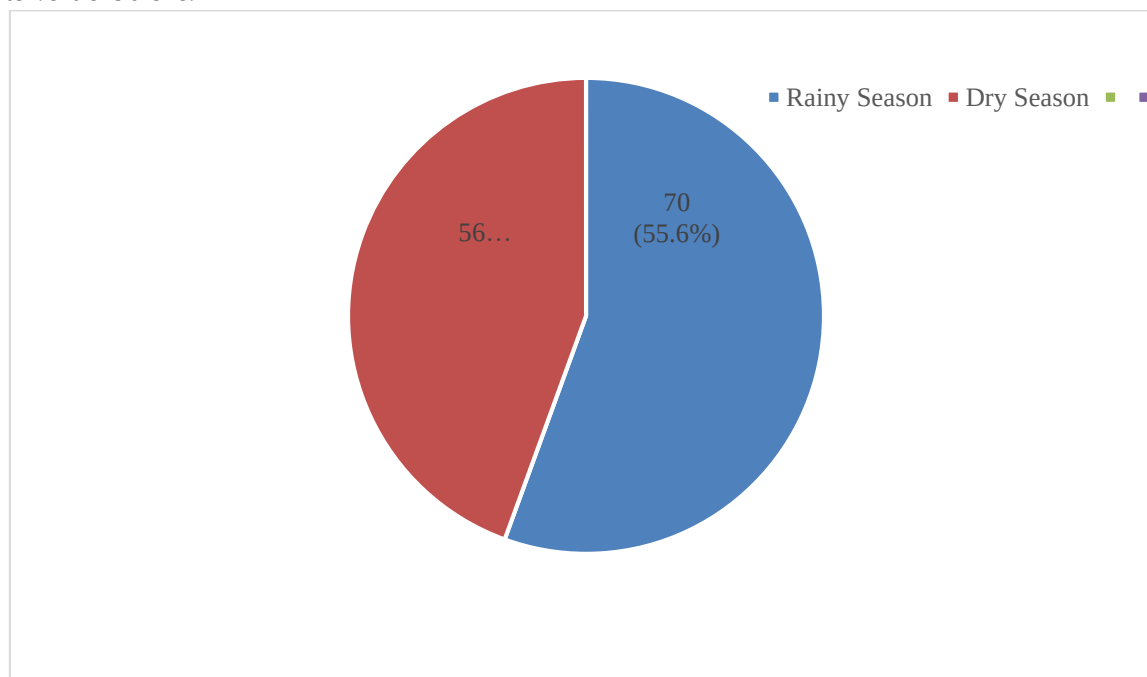


Figure 1: Seasonal Distribution of Isolates from Borehole Water Samples

Table 2 presents the percentage occurrence of bacterial isolates recovered from borehole water samples during the rainy and dry seasons. The distribution shows that enteric and water-associated bacteria were present in both seasons, although their relative proportions varied slightly, indicating that seasonality influences bacterial composition rather than completely eliminating contamination. While the rainy season generally favors higher diversity and occurrence of some organisms due to enhanced infiltration and runoff, the persistence of pathogens during the dry season suggests continuous contamination sources and possible internal colonization of borehole systems.

Escherichia coli was the most frequently isolated organism in both seasons, occurring at 27.14% in the rainy season and 25.00% in the dry season (Table 2), indicating fecal contamination of borehole water throughout the year. Its consistent presence across seasons suggests continuous input of fecal matter from sources such as nearby dumpsites, septic systems, and poor sanitation infrastructure (Sekgobela et al., 2023). The slightly higher percentage in the rainy season may reflect increased runoff and leachate infiltration, which enhances the transport of fecal organisms into groundwater. Previous studies have reported *E. coli* as a key indicator of fecal contamination in groundwater sources. For example, a study in Ibadan, southwestern Nigeria, reported *E. coli* as a common isolate from household wells and boreholes across both wet and dry periods (Alabi et al., 2024). Kumpel et al. (2017) reported higher frequency of thermotolerant coliform contamination, including *E. coli*, in borehole and other domestic water sources during the wet season compared to the dry season. However, some studies have observed season-independent persistence of *E. coli*, suggesting that groundwater contamination can remain high even in the dry season when surface runoff is reduced (Obanor et al., 2023). In rural and urban areas alike, factors such as proximity to latrines, septic leakage, and poor sanitary protection of boreholes contributed to continuous *E. coli* detection across seasons.

Vibrio cholerae showed a relatively high occurrence in both seasons, with 18.57% in the rainy season and a slightly higher 21.43% in the dry season (Table 2). The presence of *V. cholerae* in both seasons suggests that borehole water may act as a reservoir for this pathogen even in the absence of overt cholera outbreaks. A systematic review reported that the prevalence of *V. cholerae* in groundwater environments globally was approximately 26.2% (Awere-Duodu et al., 2025). Although *Vibrio* species are often associated with wet conditions, their presence in the dry season may be linked to biofilm formation within borehole systems, or

continuous contamination from organic waste (Lutz et al., 2013). This finding is particularly concerning due to the organism's association with cholera outbreaks in Nigeria (Lamichhane et al., 2024; Ogunniyi et al., 2025). Environmental *V. cholerae* strains carrying virulence genes have been isolated from water sources in Nigeria over multiple years (2008 – 2015) (Morgado et al., 2024). Onuorah et al. (2019) detected *V. cholerae* across seasons, with higher counts recorded during rainfall.

Vibrio parahaemolyticus displayed a clear seasonal pattern, with a higher occurrence during the rainy season (12.86%) compared to the dry season (7.14%). This pattern suggests that rainfall and increased organic matter influx create favorable conditions for the survival and dissemination of this organism. The reduction in its occurrence during the dry season may reflect less environmental transport and reduced nutrient availability. Research in aquatic ecosystems has shown that *V. parahaemolyticus* densities often increase during warmer and wetter periods when rainfall, nutrient input, and organic matter availability are higher, creating favorable conditions for growth and dissemination. In a tropical estuary study, the frequency of *V. parahaemolyticus* was significantly greater in wet-season samples (91.0%) than in dry-season samples (78.8%), with rainfall and runoff events contributing to higher bacterial presence through increased nutrient loads and water movement (Simma et al., 2025). Similarly, Paranjpye et al. (2015) reported that *V. parahaemolyticus* populations exhibit clear seasonal trends in a study in Pacific Northwest, USA, with peak abundances occurring during warmer months when environmental parameters such as temperature and nutrient availability are elevated.

Pseudomonas aeruginosa was detected at comparable levels in both seasons, representing 14.29% of isolates in the rainy season and 16.07% in the dry season (Table 2). The slightly higher proportion in the dry season may be attributed to the organism's ability to survive under nutrient-limited conditions and its capacity to form biofilms in borehole pipes and storage facilities. This characteristic allows *P. aeruginosa* to persist even when external contamination is reduced. Although *Pseudomonas aeruginosa* is primarily an opportunistic pathogen, it poses serious risks to individuals with compromised immunity, wounds, or burns (Sanya et al., 2023). In a densely populated urban center such as Onitsha, where borehole water is often consumed without treatment, the presence of these organisms represents a substantial risk for waterborne disease outbreaks, particularly during the rainy season (Kumpel et al., 2017; Okoye et al., 2024). Previous studies have demonstrated that *Pseudomonas* species and certain enteric bacteria readily form biofilms in water systems, making them difficult to eliminate without effective disinfection (Wingender & Flemming, 2011). This mechanism may explain the year-round detection of some bacterial species in the present study.

Salmonella spp. was also prevalent, accounting for 18.57% of isolates in the rainy season and 19.64% in the dry season (Table 2). The relatively stable occurrence across seasons suggests sustained fecal pollution and highlights the chronic nature of groundwater contamination in the study area. This pattern has been observed in similar settings elsewhere. For instance, a recent study of borehole water in urban Ibadan, southwestern Nigeria, reported *Salmonella enterica* from protected groundwater sources in both wet and dry seasons at similar detection rates, implying a lack of marked seasonality and continuous contamination likely due to poor sanitation and inadequate source protection (Rabiu et al., 2025). In contrast, research conducted in selected suburban communities of Rivers State, Nigeria, found significant seasonal variation in *Salmonella* contamination of borehole water, with higher detection in specific quarters of the year and absence in others, indicating that seasonal factors such as rainfall and runoff may influence pathogen occurrence under certain hydrogeological conditions (Alexander et al., 2025). The chronic nature of *Salmonella* contamination observed in this study aligns with broader evidence that groundwater can be persistently impacted by fecal pollution, especially in areas with inadequate sanitation and close proximity to contamination sources (Agga et al., 2023). *Salmonella* infection possesses public health significance due to its role in enteric diseases such as typhoid fever, transmitted through consumption of contaminated water (Olalemi et al., 2021). This consistency across seasons—rather than a marked seasonal spike—suggests that interventions aimed at improving sanitation infrastructure, protecting borehole integrity, and implementing routine microbial monitoring are needed year-round to effectively reduce the risk of waterborne infections.

Shigella spp. recorded the lowest overall occurrence but were present in both seasons, with 8.57% in the rainy season and 10.71% in the dry season (Table 2). Despite lower occurrence recorded in rainy season, reduced groundwater recharge during the dry season can cause concentration of existing contaminants and bacterial isolates (Danyyabu et al., 2025). In addition, bacterial persistence may be supported by biofilm formation within borehole casings and distribution pipes (Erdei-Tombor et al., 2024). Moreover, although there is lower frequency, the detection of *Shigella* spp. is significant because of their low infectious dose. Their presence indicates that even small volumes of untreated borehole water could be sufficient to cause infection.

In reference to international and national safe-water standards, the World Health Organization (WHO, 2022) and the Nigerian Standard for Drinking Water Quality (NSDWQ, 2007) stipulated that total coliforms, fecal coliforms, and pathogenic bacteria should be completely undetectable in potable water. These findings emphasize the need for year-round water treatment, routine microbial monitoring, improved regulation of borehole siting, adequate sanitation and effective waste management practices (Kumpel et al., 2017; Okoye et al., 2024).

Table 2: Percentage of Occurrence of the Bacterial Isolates

Bacterial Isolate	Rainy season (n)	Percentage Occurrence (%)	Dry season (n)	Percentage Occurrence (%)
<i>Vibrio cholerae</i>	13	18.57	12	21.43
<i>Escherichia coli</i>	19	27.14	14	25.0
<i>Vibrio parahaemolyticus</i>	9	12.86	4	7.14
<i>Pseudomonas aeruginosa</i>	10	14.29	9	16.07
<i>Salmonella</i> spp	13	18.57	11	19.64
<i>Shigella</i> spp	6	8.57	6	10.71
Total	70	100	56	100

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Conflict of interest

The Authors declare that there is no Conflict of Interest (COI) that may have affected their research.

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